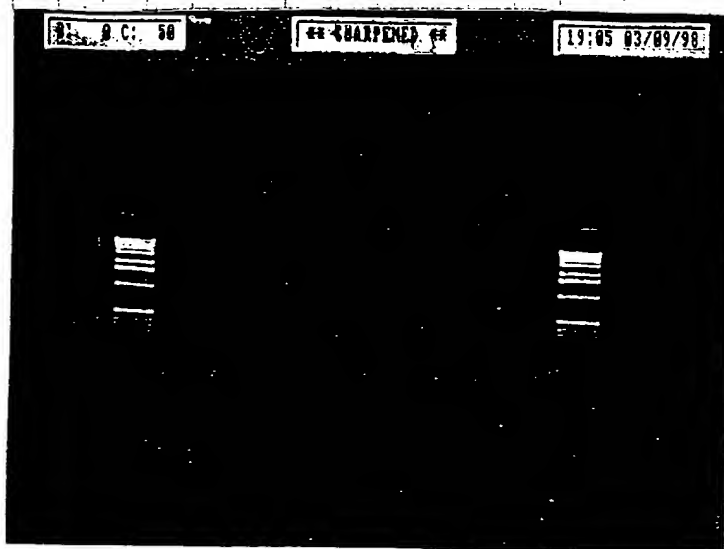
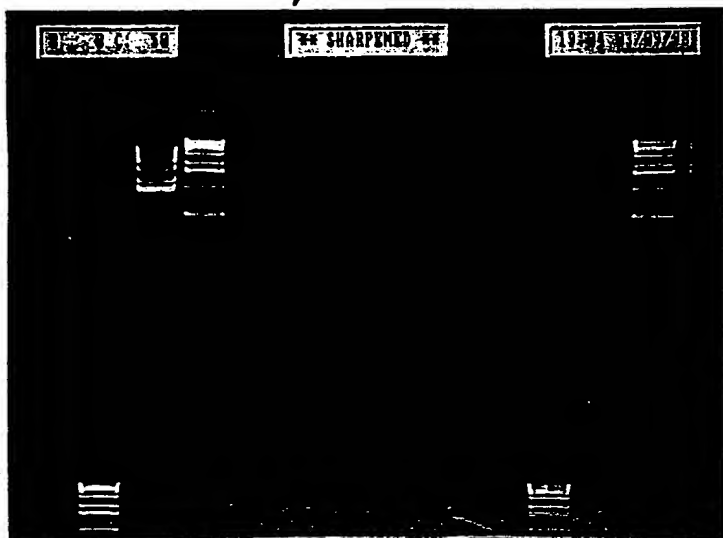


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-49665 200bp



sourcing for 49665

18
 1> 100ng ssb
 90 5> blentag buffer
 18 1> dNTP
 18 1> forward
 18 1> reverse
 18 1> blentag
 720 40> ddH₂O
 50> total

95°C 5min 1 cycle

95 1min
 57 1min
 72 1.5min

20 cycles

1.5% TAE gel

228 may possibly have the band

Thoughts try DMSO w/ amplitag and see if this helps

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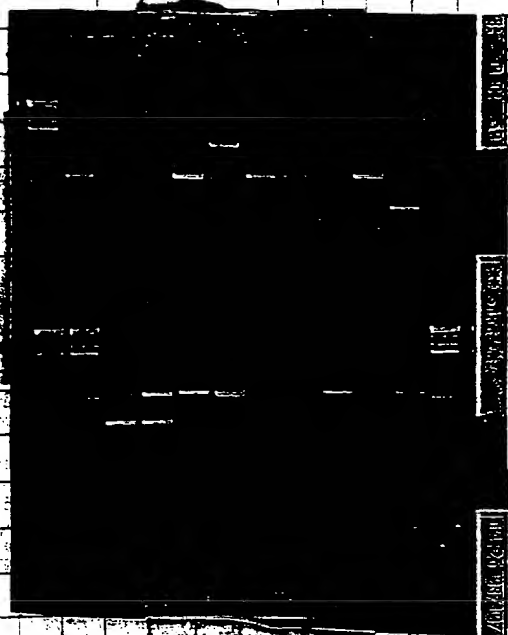
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Bethanne Dues

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PCR sourcing w/ 5% DMSO & tag

	20
1x ss lib	
5x buffer	1100
3x MgCl ₂	600 600
2.5x DMSO	55
1x forward	220
1x reverse	220
1x dNTP	220
1x amplitag	220
To 50x ddH ₂ O	759

95°C 5 min 1 cycle
 95° 1 min
 56° 1 min
 72° 1 min 25 cycles

product should be around 200bp

277 278 294 possibilities

run gel and do Southern
 order

278 if from yesterday	229D
26	229
94	230
99	247
135	247
153	255
154	293
227D	294
227	301
228	302
228D	
228	

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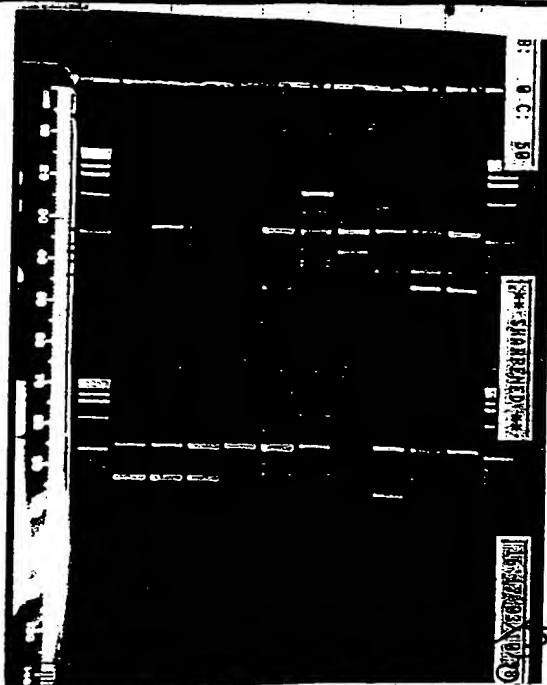
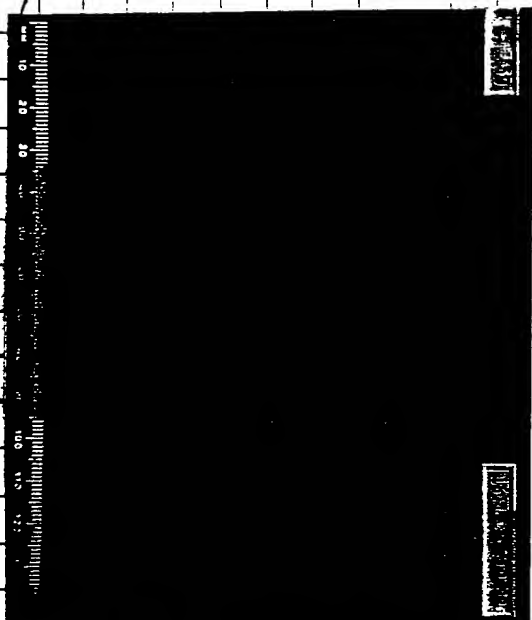
Date

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 Bethanne Zemel

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1st amps add 500x transfection + 500x compl
let rock like add 10 ml media

1 56436
5 57695
9 56350
15 57694
17 57834
4 58800

let go until Friday
then harvest cells.

Althoughts Southern for 49665 cytokine.
Probably 227 or 228 has it
If not try 254 etc

Take apart southern. Neutralize and bake
2-3 hrs in 80° vacuum oven

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Bithanne Devel

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PAGE: 1

USER: 5 ID: 32P MAXI

PRESET TIME: 1.00

PRINTER: STD

RS232: OFF

H#: YES

SCR: NO

RCM: YES

COMMENTS:

SAMPLE REPEATS: 1

REPLICATES: 1

MULTIPLIER: 1.000000

DATA CALC: CPM

COUNT BLANK: NO

VIAL SIZE: MAXI

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE: YES

SAM POS
NOTIME
MIN

H#

32P
CPM %ERROR

RCM

ELAPSED
TIME

SAM NO	POS	TIME MIN	H#	CPM	%ERROR	RCM	ELAPSED TIME
1	1-1	0.51	49.0	86389.21	0.95	0.00	1.38

make probe 49665

1x 49665 pl

10x 8³²P ATP

6x T4 pnt buffer

2x T4 pnt

41x ddH₂O

mix and incubate 30 min 37°C

clean

dilute 1:10 and count

Miniprep + and do Xba Digests

5x cDNA

2x buffer

1x Xba

12x ddH₂O

HNM402

104

32

384

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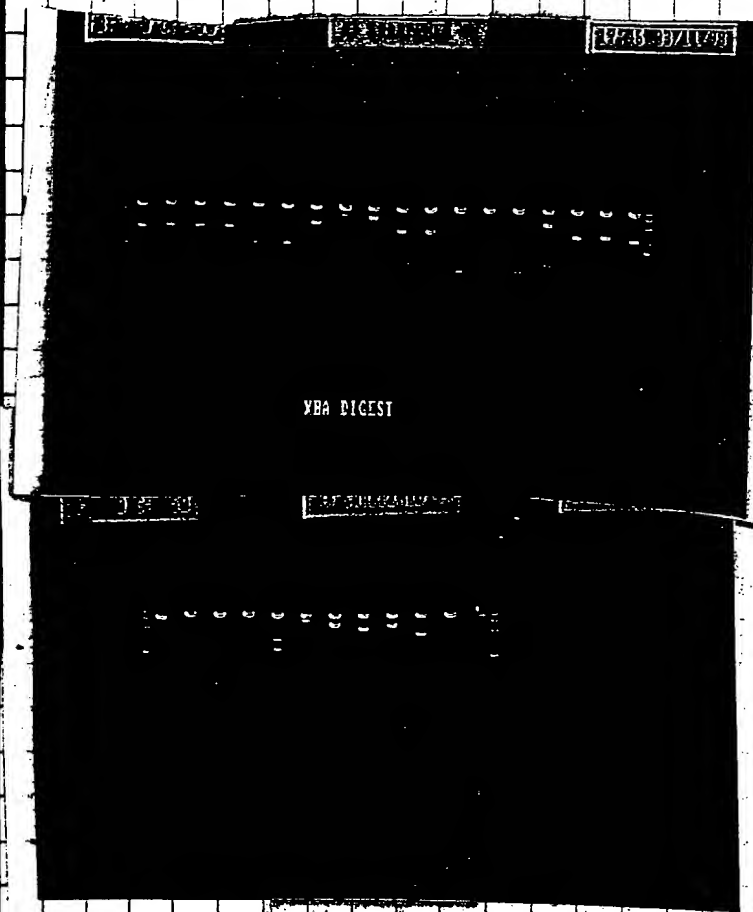
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B. Thorne Devel

Record d by

Dat

From Page N



52159

(3)

(4)

(5)

(7)

any

52721

3

(4)

(5)

52165

2 (M)

(3)

52163

(2)

(3)

any

52175

(1)

(3)

(4)

any

52035

1

(2)

(4)

(5)

any

52161

1

(2)

(7)

52772

(1)

(5)

-? replace peno

52765

(1)

(2)

(3)

52162

(1)

? replace

52160

1

42836

2

→ ?

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Inv nt d by

Bethanne Daniel

R c rded by

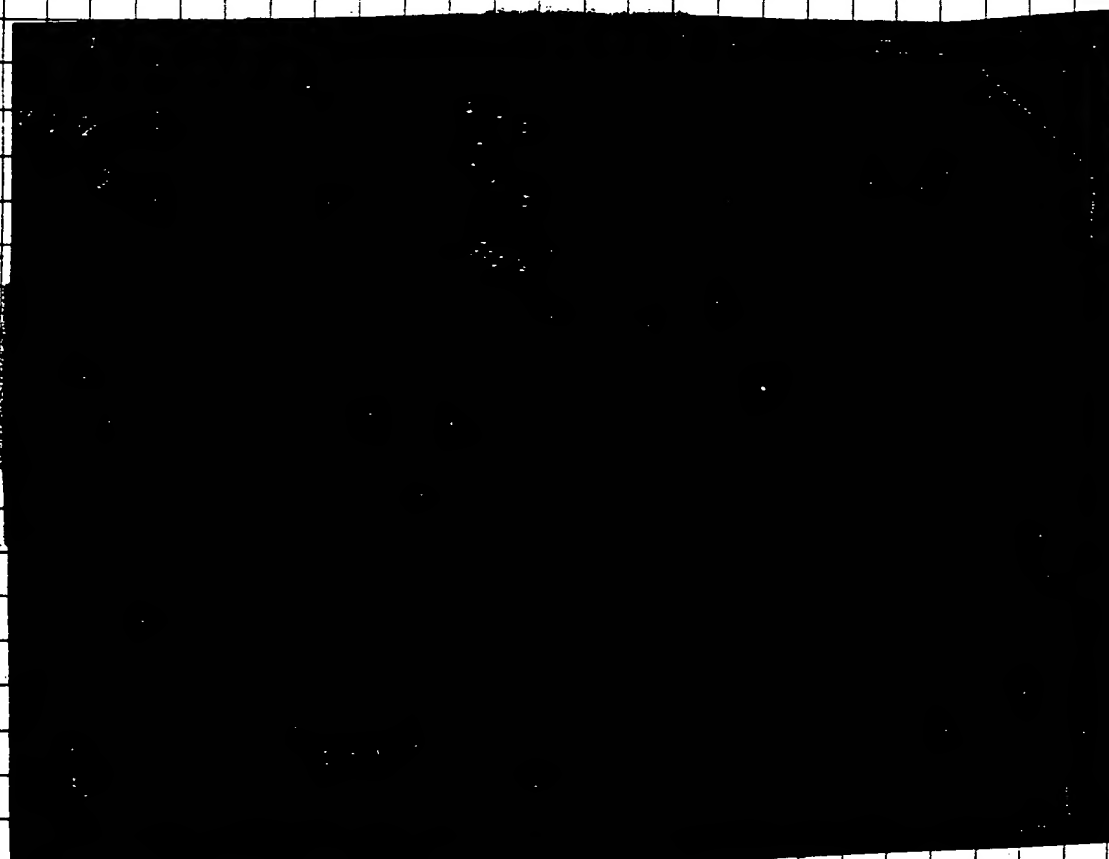
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Wash southern

2XSSC + 0.1% SDS	42°	40 min
0.5XSSC + 0.1% SDS	42°	15 min
0.1XSSC + 0.1% SDS	42°	10 min

expose to film 2 hrs develop

Source for cloning 49665
227, 228, 294

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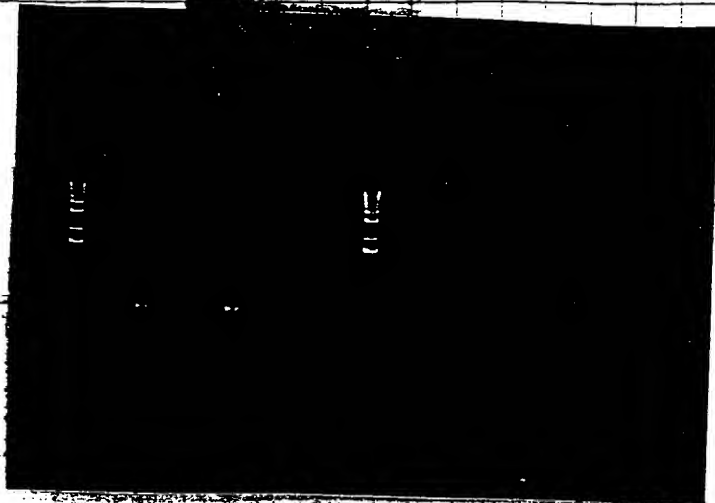
Invented by

Bethanne Deuel

Recorded by

Dr. A.

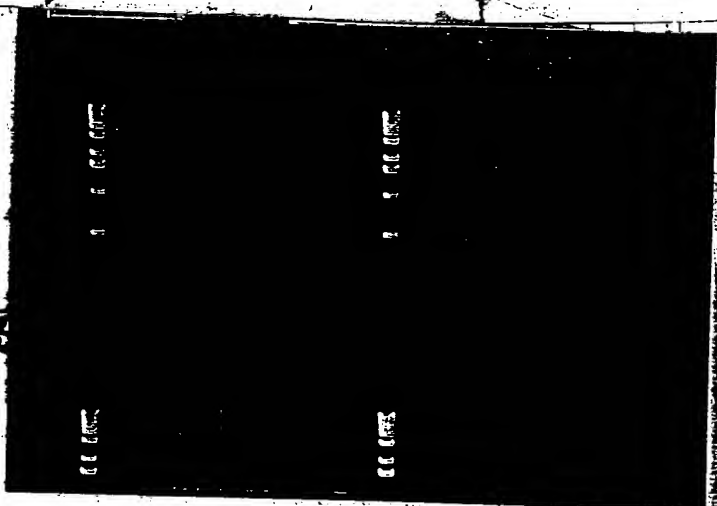
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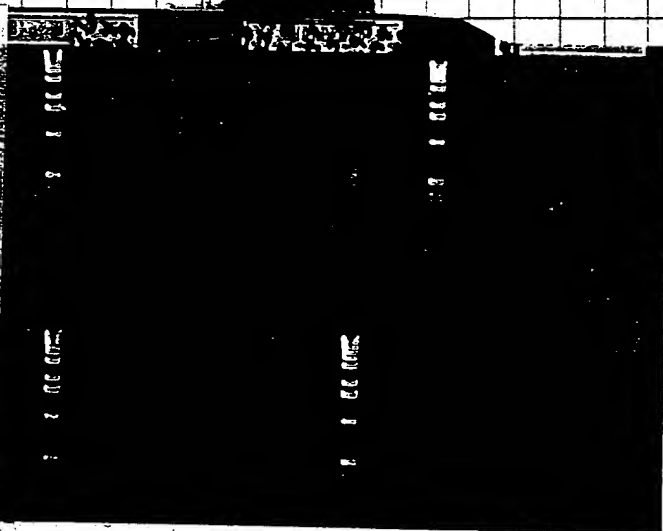
154? 247? 255?

38237

1036



38085 - 280bp 462bp



60684 ~150

Cloning

~49665 - wash buffers

2XSSC + 0.1% SDS 40 min

0.5XSSC + 0.1% SDS 20 min

let air dry expose to film develop

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Sourcing
 run PCR to check size of
 60684
 the bottom bands
 are 150

so use 216, 153, 254, or 247
 for cloning

Cloning - 1/2 pick + for 49665
 grow O/N
 $39 + \frac{216}{68} +$ in 17,000 colonies

2) Transform and plate rescues from yesterday

PCR for new sourcing

60748 - 110 lib 56° annealing

38085 - 8 lib 12 diff annealing temp

95° 5 min 1

95° 1 min

52-63° 1 min

72° 1 min 10 sec 25

72°C 5 min 1

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do miniprep on 49665

Baculovirus

set up 1st amp

Cloning

make probes for

52781	36456	36967	1097A	0.5 μ probe	12
60684	38096			3 μ pink buffer	36
60748	36461			5 μ γ 32 PATP	60
38085	35735			2 μ T4 pink	24
38237	36963			19.5 μ ddH ₂ O	234

clean probes in Purol quant G⁵⁰ micro columns
freeze

set up PCR for sourcing of 14 oligos in
12 libraries

plate in vivo from yesterday

set up PCR for sourcing of 49665

1 μ bugs	12		
5 μ γ buffer	60	—	
1 μ forward 49665	12	—	
1 μ reverse 49665	12	—	
1 μ dNTP	12	—	
1 μ γ buffer	12	✓	
40 μ ddH ₂ O	480	✓	

cycling
cond
for
broth
except
72°C 1.5 min
in a water bath

95°C 5 min 1 cycle
95°C 1 min
59 1 min
72 3 min 3 cycles
95 1
57 1
72 3 3 cycles

95°C 1 min
55°C 1 min
72°C 3 min
20 cycles

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Date

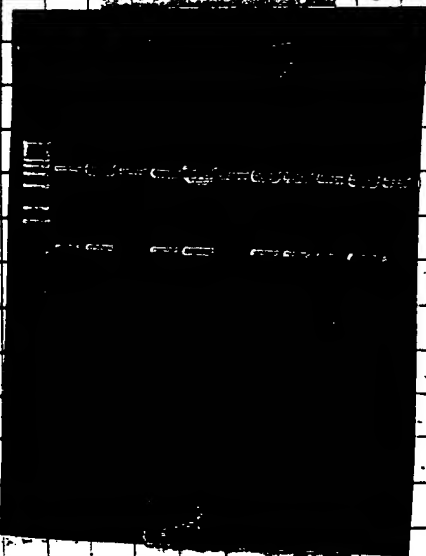
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49665 REN

Set up Xba Digest for 49665



5x CDNA	12
2x React 2	24
1x Xba I	12
12x ddH ₂ O	144

Set Digest 1 hr 37°C

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